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Enhanced 5-HT_{1A} receptor expression in forebrain regions of aggressive house mice

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Abstract

The brain 5-HT_{1A} receptor system in male wild house mice selected for high and low offensive aggression was investigated by autoradiographic analysis of in situ hybridization and radioligand binding. In high-aggressive mice, characterized by a short attack latency, the rise in plasma corticosterone concentration during the early dark phase was reduced. At that time the level of 5-HT_{1A} mRNA in the dorsal hippocampus (dentate gyrus and CA1) was twice the amount measured in low-aggressive mice that had long attack latency and high plasma corticosterone level. Increased postsynaptic 5-HT_{1A} receptor radioligand binding was found in dentate gyrus, CA1, lateral septum, and frontal cortex. No difference in ligand binding was found for the 5-HT_{1A} autoreceptor on cell bodies in the dorsal raphe nucleus. In conclusion, genetic selection for high offensive aggression co-selects for reduced (circadian peak) level in plasma corticosterone and increased postsynaptic 5-HT_{1A} receptor number in limbic and cortical regions.

Keywords: Aggression; Corticosterone; 5-HT_{1A} mRNA; 8-OH-DPAT; Hippocampus; Wild house mice

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) has been implicated in offensive, defensive and predatory aggression [30]. Recent studies indicate that in particular 5-HT_{1A} receptor ligands reduce offensive aggression [5]. Therefore, in the present study, we have measured the 5-HT_{1A} receptor expression in high- and low-offensive aggressive wild house mice with radioligand binding and in situ hybridization. These mice were genetically selected on the basis of intermale attack latency, i.e., offensive aggression against a standard opponent, in a resident-intruder paradigm [37,38]. This situation resembles the naturally occurring offensive aggression at the border of a territory [10,25]. The two mice selection lines that are available now, are characterized by high aggression as reflected in short attack latencies (SAL) in the order of seconds and by low aggression as reflected in 20- to 30-fold longer attack latencies (LAL) [37].

There is a growing body of evidence that aggressive behavior is a specific feature of a more general pattern of stress reactions displayed by animals and man in response to a changing environment. In wildlife populations individuals with an extreme difference in stress reaction co-exist. The extremes display either in response to a threat an active behavioral response (flight or fight) or a passive response (conservation-withdrawal) [11,18,20]. The active behavioral stress response pattern is associated with high sympathetic activity but low corticosteroid level, whereas during the passive behavioral stress reaction high parasympathetic activity and high corticosteroid level occur [9,20].

The SAL and LAL mice also can be seen as representatives of the above-described extremes [39]. The SAL mice display vigorous offensive aggression when confronted with an intruder, which turns into flight during confrontations with a physically stronger resident, whereas the

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low-aggressive LAL mice respond with immobility and withdrawal [8]. Corticosteroid levels are likely to be low in SAL mice and high in LAL mice. Short-term studies have shown that corticosteroids modulate 5-HT_{1A} receptor expression. Dorsal hippocampal 5-HT_{1A} receptor radioligand binding and 5-HT_{1A} mRNA hybridization are increased by adrenalectomy and decreased by corticosterone treatment [14,27,28]. Due to lower corticosterone levels in the SAL mice as compared to the LAL mice, we postulate higher 5-HT_{1A} receptor expression in the SAL mice.

The present study has been designed to measure the plasma corticosterone concentrations, the 5-HT_{1A} receptor mRNA levels and the 5-HT_{1A} receptor radioligand binding sites in the central nervous system of the high-aggressive SAL mice and the low-aggressive LAL mice. We report here that the male SAL mice display both an increased level of 5-HT_{1A} receptor mRNA and an increased number of 5-HT_{1A} receptor binding sites in the dorsal hippocampus as compared to the LAL mice.

2. Materials and methods

2.1. Mice

The lines, genetically selected for attack latency, originated from a colony of wild house mice (*Mus musculus domesticus*) maintained at the University of Groningen, The Netherlands, since 1971. The SAL males ($n = 11$) came from the 50th generation of selection; the LAL males ($n = 11$) from the 25–27th generation. The mice were housed in Plexiglass cages ($17 \times 11 \times 13$ cm) in a room with an artificial 12:12 h light/dark cycle (lights on from 00.30 to 12.30 h). Standard laboratory chow and water were available ad libitum. The mice were weaned at 3–4 weeks of age, and were paired male-female at the age of 6–8 weeks. At the age of 4 months the mice were decapitated under halothane anesthesia (no longer than 3 min) between 14.00–17.00 h (in the first half of the dark phase). Trunk blood was collected for the determination of plasma corticosterone levels. The brains were removed rapidly from the skull, immediately frozen on powdered dry ice and stored at −80°C.

2.2. Aggression test

The aggression test has been extensively described by Van Oortmerssen and Bakker [37]. Briefly, genetically selected SAL and LAL mice at the age of 92–100 days are confronted with a standard non-aggressive opponent in a neutral cage. The time it takes before a SAL or LAL mouse attacks the non-aggressive opponent is measured on three consecutive days. The attack latency score is the mean of these daily scores. Neither SAL nor LAL mice experience a social defeat. Attack latency is a reliable indicator of aggression, since there is a significantly negative correlation between attack latency and the number of attacks and accumulated attack time, including chasing, biting and fighting [12,40].

2.3. Hormones

Blood samples were immediately transferred to chilled (0°C) centrifuge tubes containing 0.01% EDTA as antioxidant and 10 μl heparin solution (500 IU/ml) as anticoagulant. Blood was centrifuged at 4°C for 10 min at 5000 rpm and 100 μl of the supernatant were stored at −20°C for corticosterone measurement. Plasma corticosterone was measured by reversed-phase high-performance liquid chromatography [16].

2.4. Riboprobes

PCR1000 plasmids containing a 350-basepair-long insert coding for the third cytoplasmatic loop of the rat 5-HT_{1A} receptor (amino acids 220–345; [1]) in two orientations, were provided by Organon International (Oss, The Netherlands). The rat sequence shows 93% homology with the corresponding part of the mouse 5-HT_{1A} receptor gene (L. Tecott, pers. commun.). These plasmids were linearized with *Eco*RI and a [^{35}S]U-labeled riboprobe was made by in vitro translation with T7 polymerase, using a standard protocol.

2.5. In situ hybridization

Brain sections of 20 μm were cut in a cryostat and thaw-mounted on poly-L-lysine coated slides. These slides were stored at −80°C until hybridization. The protocol was described before [27]. The sections were postfixed in a 4% paraformaldehyde solution for 60 min at room temperature, pH 7.4. Then the slides were rinsed twice in phosphate-buffered saline (PBS) for 5 min (room temperature), treated with proteinase K for 10 min at 37°C (1 μg/ml in 0.1 M Tris, pH 8.0), rinsed briefly in water, treated with 0.25% acetic anhydride in triethanolamine (0.1 M, pH 8.0) for 10 min at room temperature and finally rinsed in $2 \times$ SSC (SSC = 0.15 M NaCl and 0.015 M sodium citrate) for 10 min at room temperature. Subsequently the sections were dehydrated through a graded series of alcohol and air dried. A hybridization mix was prepared containing 70% deionized formamide, 10% dextran sulphate, 0.06 M phosphate buffer, $3 \times$ SSC, $1 \times$ Denhardt's solution, 10 mM dithiothreitol, 0.1 mg/ml yeast tRNA and 0.1 mg/ml sheared salmon sperm DNA. The probe was diluted to a concentration of 40×10^6 dpm/ml and then 100 μl of the mix (containing 4×10^6 dpm of probe) were applied to every slide, each containing at least eight tissue sections. Standard 24×50 mm microscopic coverslips were put on the slides that were then placed in a moist chamber and hybridized overnight at 53°C. As a control, a few slides were hybridized with a sense probe. The coverslips were removed next day and the slides were washed in $2 \times$ SSC at room temperature for 10 min, treated with

RNAse A at 37°C for 10 min (2 mg/100 ml in 0.5 M NaCl, 0.01 M Tris, pH 7.5), and washed at 55°C in 2 × SSC (10 min), 1 × SSC (10 min) and 0.1 × SSC (60 min). The slides were dried in an alcohol series, air dried, and placed under X-OMAT AR film for 2 weeks.

2.6. Ligand binding

[^{3}H]8-OH-DPAT binding was performed according to published methods with some minor modifications [33]. Briefly, slide-mounted tissue sections were preincubated in 0.17 M Tris-HCl (pH 7.6) containing 4 mM $CaCl_2$ and 0.01% ascorbic acid for 30 min at room temperature. Subsequently, sections were incubated with 0.5 nM [^{3}H]8-OH-DPAT for 60 min at room temperature. Postincubation slides were washed in incubation buffer (2 × 15 min) at 4°C and dried in a stream of cold air. Nonspecific binding was determined in the presence of 1 μM 5-HT. Sections were apposed to tritium-sensitive Amersham Hyperfilm and exposed at room temperature for 2 months.

2.7. Densitometric analysis

Autoradiograms from both in situ hybridization and in vitro receptor autoradiography were quantified using an automatic image analysis system (IBAS). After shading and background correction the optical density (OD) of the hybridization signal of 4 to 5 sections per animal was measured in the dorsal hippocampus (pyramidal cell layer of CA1 and granular cell layer of dentate gyrus) of SAL mice ($n = 5$) and LAL mice ($n = 5$). [^{3}H]8-OH-DPAT binding of 8 to 10 sections per animal was measured in the dorsal raphe nucleus, dorsolateral septum, frontal cortex and in dendritic fields of the pyramidal neurons of CA1 in the stratae radiatum and oriens, and in all fields of dentate gyrus of the SAL group ($n = 6$) and LAL group ($n = 6$).

2.8. Statistics

Behavioral data (attack latency scores) were analyzed using the Mann-Whitney U-test. Neuroendocrine data, [^{3}H]8-OH-DPAT binding and in situ hybridization data in SAL and LAL mice were analyzed using Student's t-tests.

3. Results

3.1. Attack latency

Table 1 shows, as expected, that the attack latency score of the SAL group was the lowest (fast attackers) whereas

Table 1
Attack latency, plasma corticosterone (CORT) levels and 5-HT_{1A} receptor mRNA abundance, in the CA1 and dentate gyrus (DG) of the dorsal hippocampus, in high-aggressive (SAL) and low-aggressive (LAL) mice

	Attack latency (s)	Plasma CORT (μg/dl)	Dentate gyrus arbitrary units	CA1 arbitrary units
LAL	561.1 ± 26.1	14.8 ± 1.6 [b]	0.156 ± 0.006	0.402 ± 0.024
SAL	23.8 ± 4.9[a]	6.0 ± 1.5	0.292 ± 0.040[a]	0.812 ± 0.045 [a]

Values (5 animals per group) are given ± S.E.M.
[a] At least $P < 0.05$, significantly different from LAL; [b] at least $P < 0.05$, significantly different from SAL.

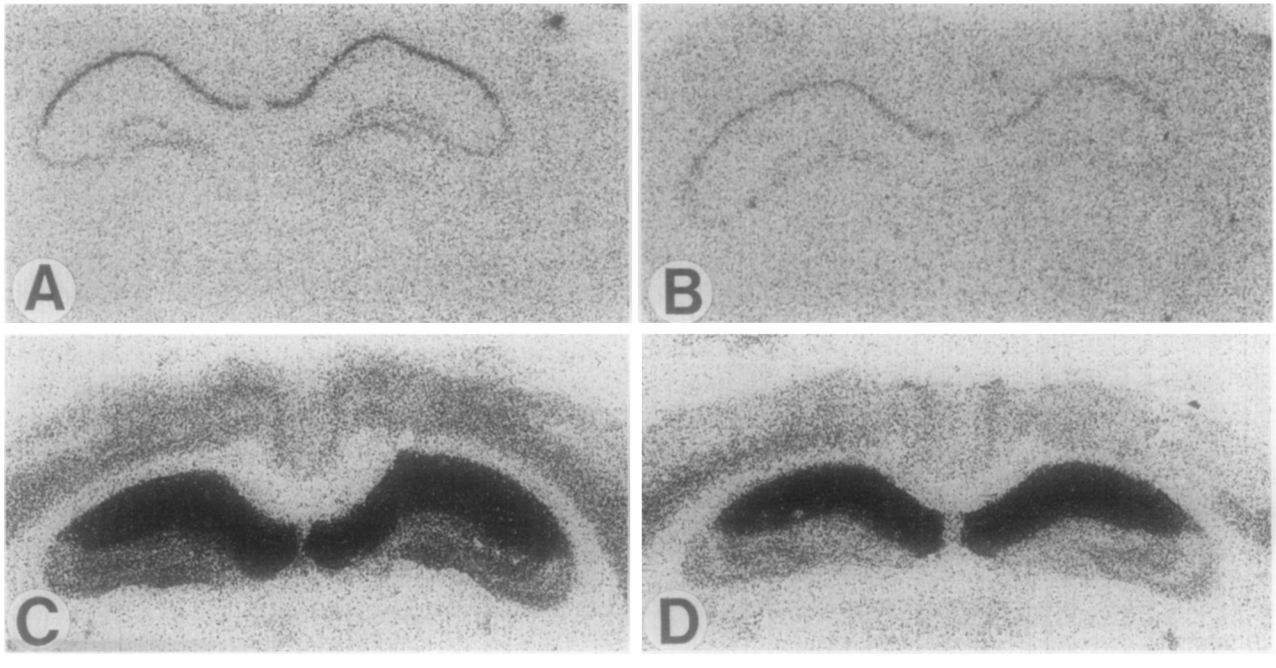

Fig. 1. Expression of hippocampal 5-HT_{1A} receptor mRNA in high-aggressive SAL (A) and low-aggressive LAL mice (B) and [^{3}H]8-OH-DPAT binding sites in SAL (C) and LAL mice (D).

the LAL group reached the highest scores (slow attackers). The attack latency score of the SAL group was significantly different from the LAL group ($P = 0.0079$).

3.2. Corticosterone levels

Table 1 shows that the plasma corticosterone level in LAL mice was significant higher than in SAL mice in the early dark phase ($P = 0.0005$).

3.3. In situ hybridization

In situ hybridization of the 5-HT_{1A} receptor transcript gave a specific signal with very high density in the CA1 and dentate gyrus of the dorsal hippocampus. In Fig. 1A and B, representative sections of the SAL and LAL groups are shown. Table 1 shows that in SAL mice the level of 5-HT_{1A} mRNA in the dentate gyrus was 89% higher, and in the CA1 102% higher than in LAL mice ($P = 0.010$ and $P < 0.0001$, respectively).

3.4. Ligand binding

Fig. 1C and D shows representative sections of SAL and LAL mice with specific 5-HT_{1A} receptor ligand binding in dorsal hippocampus. Table 2 shows that [^{3}H]8-OH-DPAT binding was 9–28% higher in the SAL group as compared to the LAL group in forebrain areas. The [^{3}H]8-OH-DPAT binding in SAL mice was significantly higher in the dentate gyrus ($P = 0.011$), CA1 ($P = 0.049$), lateral septum ($P = 0.042$), and frontal cortex ($P = 0.0078$) than in the LAL line. No difference in ligand binding was found in the dorsal raphe nucleus.

4. Discussion

In the present study we found that male wild house mice (*Mus musculus domesticus*), which were genetically selected for attack latency, showed a distinct relationship between corticosteroid levels and brain 5-HT_{1A} receptor expression. The high-aggressive SAL mice showed lower plasma corticosterone levels than the low-aggressive LAL mice. Their 5-HT_{1A} mRNA level in dentate gyrus and CA1 cell field of the hippocampus was much higher. Moreover, an increased number of postsynaptic [^{3}H]8-OH-DPAT-labeled binding sites was found in limbic and cortical structures in the high-aggressive group as compared to the low-aggressive group. No difference in density was found for the 5-HT_{1A} autoreceptors on cell bodies in the dorsal raphe nucleus.

The observed differences in hypothalamus-pituitary-adrenocortical activity in SAL and LAL mice are in agreement with previous findings. Individuals with an active behavioral response (flight or fight), resembling SAL mice, or with a passive behavioral stress reaction (conservation-withdrawal), resembling LAL mice, have respectively low and high corticosteroid levels [9,18,20,24]. The difference in 5-HT_{1A} receptor expression in low and high aggressive mice may be ascribed to the action of corticosterone on the 5-HT system. Corticosterone affects 5-HT neurotransmission via different mechanisms. First, corticosteroids via glucocorticoid receptors (GRs) in the raphe nuclei increase the efficiency of tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5-HT and consequently its release in terminal areas [2–4]. Thus, the relatively lower corticosterone levels in aggressive mice may lead to a lower 5-HT biosynthesis and release. Second, the postsynaptic 5-HT_{1A} receptor-mediated hyperpolarization response of hippocampal neurons is blocked by low levels of corticosterone acting predominantly through binding to mineralocorticoid receptors (MRs). Higher levels of corticosterone reverse this MR-mediated inhibition by activation of colocalized (GRs) [21–23]. Third, corticosteroids may exert via MRs or GRs a downregulation of the postsynaptic 5-HT_{1A} receptor expression. Both 5-HT_{1A} mRNA hybridization and 5-HT_{1A} receptor radioligand binding are increased in CA1 and dentate gyrus of the hippocampus after adrenalectomy (ADX). Low levels of corticosterone or aldosterone reverse these effects of ADX and strongly suggest the involvement of MRs [14,27,28,36]. In contrast, ADX exerts only minor, but opposite effects on 5-HT_{1A} autoreceptors in the dorsal raphe nucleus [17,36]. In addition, in tree shrews, long-term social stress enhances the plasma corticosteroid level and reduces 5-HT_{1A} receptor expression in the dorsal hippocampus, but not in the dorsal raphe nucleus [19]. In the present study the relatively enhanced postsynaptic 5-HT_{1A} receptor expression in limbic and cortical structures in SAL mice would be predicted on the basis of the lower circulating corticosterone levels in these mice.

We hypothesize that this enhanced receptor expression

Table 2
[^{3}H]8-OH-DPAT binding sites in dorsal raphe nucleus, dorsal hippocampal dentate gyrus and CA1, lateral septum and frontal cortex in high-aggressive (SAL) mice and in low-aggressive (LAL) mice

	Dorsal raphe	Dentate gyrus	CA1	Lat. septum	Frontal cortex
LAL	0.571 ± 0.024	0.271 ± 0.015	0.674 ± 0.027	0.431 ± 0.022	0.308 ± 0.015
SAL	0.568 ± 0.008	0.347 ± 0.021[a]	0.735 ± 0.004[a]	0.499 ± 0.017[a]	0.394 ± 0.021 [a]

Values (6 animals per group) are given in arbitrary units ± S.E.M.
[a] At least $P < 0.05$, significantly different from LAL.

is not limited to offensive aggression, but is also related to a more general pattern of active behavioral stress reaction. It is suggested that high aggressive individuals and animals that prefer an active behavioral strategy are a different behavioral expression of the same genotype. For example, SAL mice display more active avoidance, more defensive burying and more nestbuilding than LAL mice [6–8,35]. Conversely, rats selected for high active avoidance and mice selected for high nestbuilding are both highly aggressive as compared to their passive counterparts [32,34]. This implies that animals selected for high scores of active avoidance, nestbuilding or defensive burying also are predicted to have low corticosterone levels and enhanced postsynaptic 5-HT_{1A} receptor expression.

The question whether increased 5-HT neurotransmission is the causal factor for offensive aggression is yet still difficult to answer. Recently, a role for 5-HT in offensive aggression was suggested from experiments with transgenic mice lacking monoamine-oxydase or the 5-HT_{1B} gene expression. Both types of knock-out mice have increased offensive behavior [13,31]. Several studies suggest that increased 5-HT neurotransmission is required for aggressive behavior. It has been shown that 5-HT_{1A} receptor agonists, via stimulation of autoreceptors, inhibit both 5-HT release and offensive aggression [5,29]. It can not be excluded that the reduction in offensive aggressive behavior by 5-HT_{1A} receptor agonists is due to an anxiolytic action on behavior, thereby attenuating offensive behavior [5]. In addition, transgenic mice deficient for α-calcium kinase II exhibit a reduction in 5-HT release, a decrease in anxiety and a decrease in offensive behavior [15]. However, many studies have shown opposite results, i.e., a general enhancement of 5-HT neurotransmission that attenuates aggression in animals [26,30]. Methodological differences such as acute, chronic or genetic studies and the differences in time period between 5-HT depletion and behavioral testing may underlie some of the conflicting data.

In conclusion, genetic selection for short attack latency, i.e., high offensive aggression, co-selects for a relative low corticosteroid hormone level, but high forebrain 5-HT_{1A} receptor expression. Further studies in these aggressive mice are planned to investigate whether chronic elevated corticosterone levels reduce both the 5-HT_{1A} receptor expression and the offensive aggression.

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